Comparative Sperm Structure in Bats (Chiroptera): Some Taxonomic and Adaptive Implications

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ABSTRACT

In Megachiroptera, the spermatozoa of Pteropodidae have a wide spoon-shaped head, a large acrosome, a rounded nucleus and a tail with a long midpiece. In Microchiroptera, the spermatozoa of Rhinolophidae have a spatulate head with a large acrosome, being generally uniform in morphology at the specific level, but the midpiece varies in length with species. In Vespertilionidae examined, two subfamilies of Vespertilioninae and Murininae are separable from another subfamily Miniopterinae in having a small acrosome and long midpiece. Furthermore, in the vespertilionine bats, greater uniformity in sperm structure prevails in the tribe Myotini (*Myotis*) and the tribe Plecotini (*Plecotus* and *Barbastella*) when compared with the tribe Pipistrellini (*Pipistrellus*, *Nyctalus* and *Vespertilio*). The sperm nucleus of the tribe Myotini *s. lat.* (*Myotis*, *Plecotus* and *Barbastella*) is longer than that of Pipistrellini, and the midpiece is obviously distinctive at the generic and specific levels. The midpiece in *Myotis nattereri*, *Myotis macrodactylus* and *Pipistrellus abramus* is longer than that in the other vespertilionine bats examined, and their mitochondria are large in number and in size. The present study demonstrates the potential usefulness of sperm morphology in taxonomic studies of Chiroptera. In addition, the presence of a well-developed midpiece with a unique arrangement of abundant, large mitochondria in spermatozoa of hibernating bats is explained as an adaptive phenomenon for prolonged survival of spermatozoa.

RÉSUMÉ

Structure comparée du spermatozoïde chez les Chauve-souris (Chiroptera): quelques implications pour la taxonomie et l'adaptation

Chez les Megachiroptera, le spermatozoïde des Pteropodidae a une tête large en forme de cuillère, un grand acrosome, un noyau arrondi et une queue avec une pièce intermédiaire longue. Chez les Microchiroptera, le spermatozoïde des Rhinolophidae a une tête spatulée avec un grand acrosome qui est généralement de morphologie uniforme au niveau spécifique, mais la longueur de la pièce intermédiaire varie selon les espèces. Chez les Vespertilionidae qui ont été examinés, les deux sous-familles des Vespertilioninae et Murininae peuvent être distinguées de l'autre sous-famille Miniopterinae grâce à leur petit acrosome et leur longue pièce intermédiaire. De plus, chez les Vespertilioninae, l'uniformité de la structure du spermatozoïde est plus profonde dans les tribus Myotini (*Myotis*) et Plecotini (*Plecotus* et *Barbastella*) que dans la tribu Pipistrellini (*Pipistrellus*, *Nyctalus* and *Vespertilio*). Le noyau du spermatozoïde dans la tribu Myotini *s. lat.* (*Myotis*, *Plecotus* et *Barbastella*) est plus long que celui des Pipistrellini, et la pièce intermédiaire permet de distinguer de manière évidente les genres et les espèces. Chez *Myotis nattereri*, *Myotis macrodactylus* et *Pipistrellus abramus*, la pièce intermédiaire est plus longue que celle des autres Vespertilioninae examinés et les mitochondries sont grandes et nombreuses. Cette étude démontre l'utilité potentielle de la morphologie du spermatozoïde pour les études taxonomiques sur les Chiroptera. De plus, la présence d'une pièce intermédiaire bien développée avec une disposition originale de mitochondries grandes et abondantes chez les chauve-souris qui hibernent est expliquée comme un phénomène adaptatif pour prolonger la survie des spermatozoïdes.

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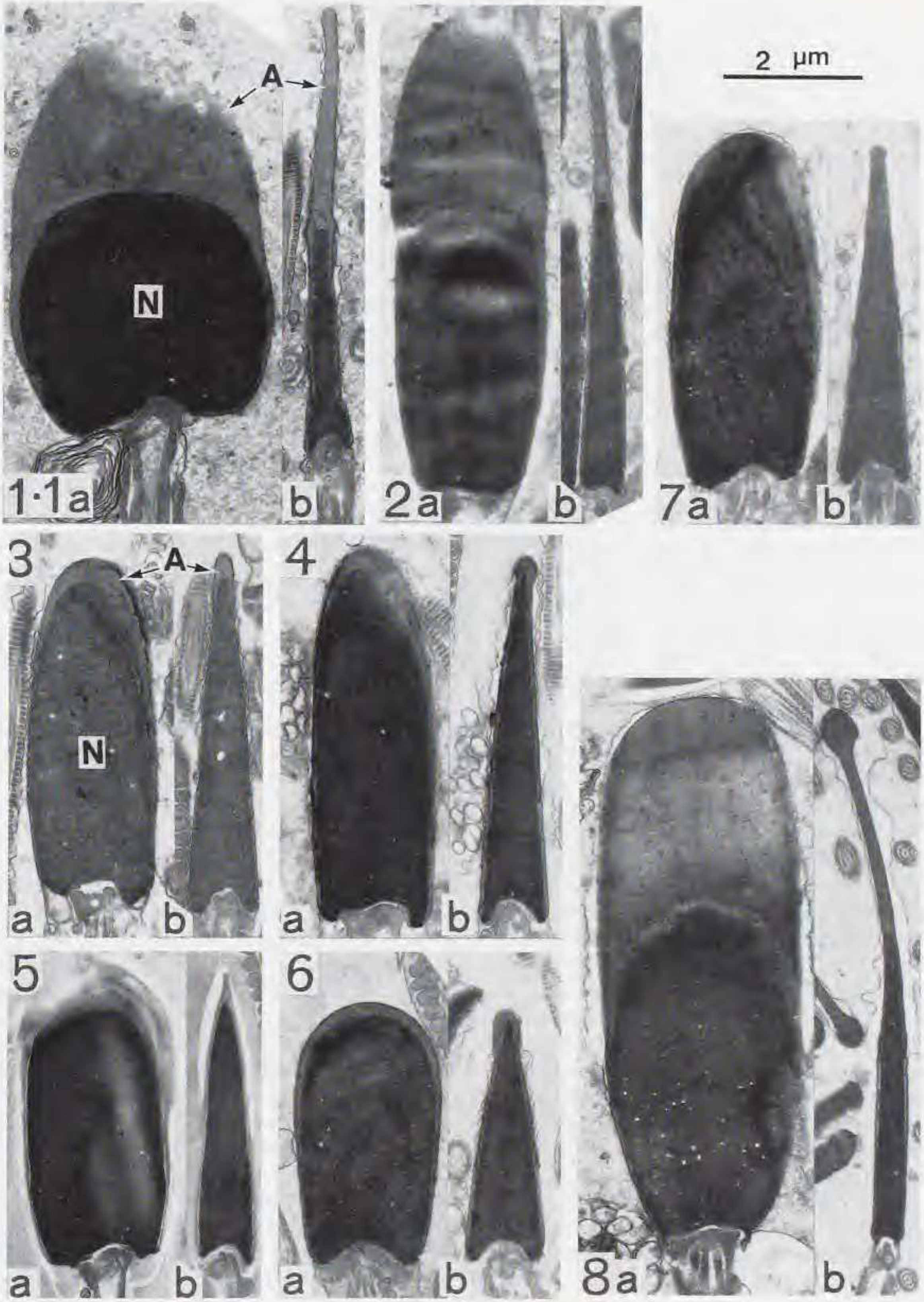
The phylogenetic system must be built up on conclusions obtained from various fields of study. The morphology of spermatozoa is believed to be a good tool for better understanding of phylogenetic relationships, because of their conservatism against environmental factors. In this connection, there have been some light microscopical studies on the morphology of chiropteran spermatozoa [2-5]. On the other hand, electron microscopic studies have been made to analyse sperm survival mechanisms in hibernating bats from the viewpoint of physiological adaptation [12, 17, 19, 22]. The ultrastructure of the sperm of pteropodids has also been described [18]. The aim of the present study is to examine with the electron microscope the comparative morphology of epididymal spermatozoa in 25 bat species (Table 1), and to discuss some taxonomic and adaptive implications of sperm fine structure.

TABLE 1. — Species examined.

Suborder	Family	Subfamily, Tribe	Species	Locality
Megachiroptera	Pteropodidae	Pteropodinae	*Pteropus dasymallus* *Cynopterus brachyotis*	Japan Malaysia
		Macroglossinae	*Macroglossus minimus* *Eonycteris spelaea*	Malaysia Malaysia
Microchiroptera	Rhinolophidae	Rhinolophinae	*Rhinolophus cornutus* *Rhinolophus monoceros* *Rhinolophus imaizumii* *Rhinolophus ferrumequinum* *Rhinolophus luctus*	Japan Taiwan Japan Japan Taiwan
	Vespertilionidae	Vespertilioninae Myotini	*Myotis adversus* *Myotis nattereri* *Myotis hosonoi* *Myotis macrodactylus* *Myotis formosus* *Myotis lucifugus*	Taiwan Japan Japan Japan Korea Canada
		Plecotini	*Plecotus auritus* *Barbastella leucomelas*	Japan Japan
		Pipistrellini	*Pipistrellus endoi* *Pipistrellus javanicus* *Pipistrellus abramus* *Pipistrellus avii* *Nyctalus noctula* *Vespertilio superans*	Japan Indonesia Japan Korea Japan Japan
		Murininae	*Murina leucogaster*	Japan
		Miniopterinae	*Miniopterus schreibersii*	Japan

FIG. 1. — Electron micrographs of sperm heads of bat species (a, frontal section; b, sagittal section). The sperm head of Pteropodidae has a spoon-shaped head, a large acrosome (A) and a rounded nucleus (N). The spermatozoa of Rhinolophidae have a spatulate head with a large acrosome. In Vespertilionidae, two subfamilies of Vespertilioninae and Murininae are separable from another subfamily Miniopterinae in having a small acrosome. **1:** *Pteropus dasymallus*; **2:** *Rhinolophus cornutus*; **3:** *Myotis macrodactylus*; **4:** *Barbastella leucomelas*; **5:** *Pipistrellus abramus*; **6:** *Vespertilio superans*; **7:** *Murina leucogaster*; **8:** *Miniopterus schreibersii*.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MATERIAL AND METHODS

The order Chiroptera is divided into two suborders; Megachiroptera, including a single family Pteropodidae, and Microchiroptera, consisting of the remaining families. Bat specimens examined were as follows: four genera and four species representing the family Pteropodidae, one genus and five species of the family Rhinolophidae, and eight genera and sixteen species of the family Vespertilionidae (see Table 1). The cauda epididymidis of each species was fixed in cold 3% glutaraldehyde buffered with 0.1 M-phosphate buffer (PBS) at pH 7.4, then rinsed thoroughly with the same PBS and postfixed in cold 1.3% osmium tetroxide in the PBS, dehydrated with alcohol and embedded in Epon 812 (Taab). Sections (60 nm) were doubly stained with uranyl acetate and lead citrate before examination in an Hitachi HS-9 or an Hitachi H-600A electron microscope (75 kV).

OBSERVATIONS

In Megachiroptera, the *Pteropus dasymallus* spermatozoon, when cut frontally, has a dorsoventrally flattened, wide spoon-shaped head with convex lateral contours. The head is 6 µm in length, of which the posterior 3.2 µm is occupied by the rounded nucleus, being about 3.4 µm in width (Fig. I 1a). When cut sagittally, the nucleus has a convexity in the posterior region, and the acrosome closely fits into an anterior concavity; thus, the nucleus appears rocket-shaped, being thicker at the base, and tapering to the tip (Fig. I 1b). The acrosome protrudes for a length of 2.8 µm from the tip of the nucleus. The equatorial segment of the acrosome occupies the posterior one-fourth of the nucleus. Thus, the postacrosomal region is shorter than that in Microchiroptera (cf. Fig. I 2-8). The inner acrosomal membrane is serrated, while it is smooth in Microchiroptera. The sperm tail is very long, because of the conspicuously long midpiece with a length of 22.0 µm (Fig. II 1a, b). Mitochondria are large in number, but small in size (Fig. II 1a, 3a). The arrangement of mitochondria in *Pteropus* is similar to that in common mammalian species, in which mitochondria are disposed in a helix with their tapering ends overlapping or abutting end-to-end. The characteristics of the *Pteropus dasymallus* spermatozoon resemble those of the other three genera examined in Pteropodidae (Fig. II 1b).

In Rhinolophidae, of the Microchiroptera, the spermatozoon of *Rhinolophus cornutus* has a dorsoventrally flattened, spatulate head with parallel sides and a narrow nucleus. The nucleus occupies 4.1 µm of the head with a total length of 6.8 µm, being about 2.1 µm in width (Fig. I 2a). When cut sagittally, the nucleus appears extremely slender and wedge-shaped, being thicker at the base, and tapering to a point (Fig. I 2b). The large acrosome extends anteriorly beyond the leading edge of the nucleus for a length of 2.7 µm. The equatorial segment of the acrosome is situated at about the middle portion of the nucleus (Fig. I 2b). Thus, the postacrosomal region is longer than that in Megachiroptera. The midpiece (12.0 µm) in this species is very short compared with the midpiece (15.0 µm) with abundant mitochondria in *R. ferrumequinum* (Fig. II 2a, d). In *Rhinolophus*, the sperm head is generally uniform in morphology at the specific level, but the midpiece varies in length with the species (Fig. II 2a-e).

In Vespertilionidae, two subfamilies of Vespertilioninae and Murininae are separable from the subfamily Miniopterinae in having a small acrosome and long midpiece. Furthermore, in the vespertilionine bats examined, greater uniformity in sperm structure prevails in the tribe Myotini (*Myotis*) and the tribe Plecotini (*Plecotus* and *Barbastella*) when compared with the tribe Pipistrellini (*Pipistrellus*, *Nyctalus* and *Vespertilio*). The sperm nucleus of the tribe Myotini *s. lat.* (*Myotis*, *Plecotus* and *Barbastella*) is longer than that of Pipistrellini (Fig. I 3-6). For example, the sperm nucleus occupies 4.9 µm of the head (5.1 µm in length) in *Myotis macrodactylus*, and 3.7 µm of the head (4.0 µm in length) in *Pipistrellus abramus*. At the specific level, the spermatozoa of *Pipistrellus endoi* and *P. javanicus* have a narrower head and shorter midpiece than the *P. abramus* spermatozoon.

In Vespertilionidae, the midpiece is clearly distinctive at the generic and specific levels. The midpiece in *Myotis nattereri*, *Myotis macrodactylus* and *P. abramus* is longer than that in the other vespertilionine bats examined, and their mitochondria are large in number and in size (Fig. II 3-6). The arrangement of mitochondria is such that each turn of the flat helix is made up of just two

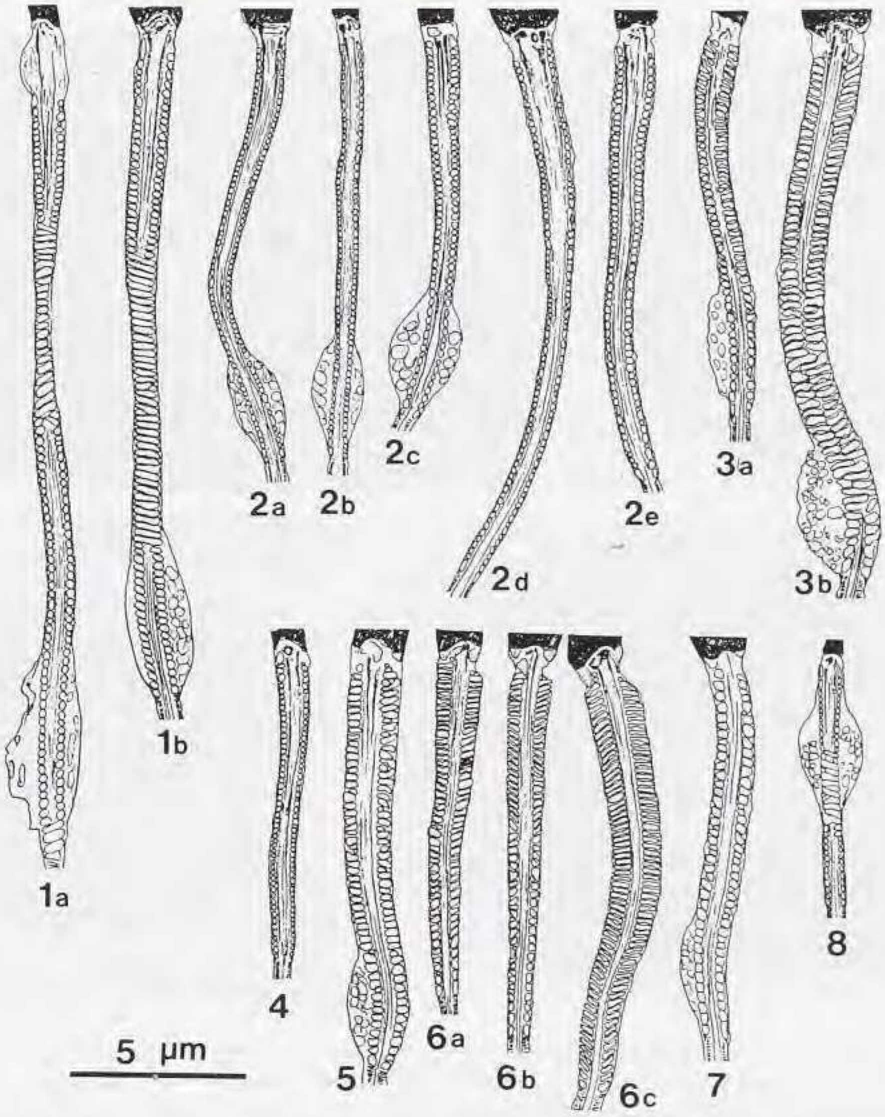

FIG. II. — Semischematic reconstruction of the midpiece in bats, based on electron microscopic observations. **1a:** *Pteropus dasymallus*; **1b:** *Macroglossus minimus*; **2a:** *Rhinolophus cornutus*; **2b:** *Rhinolophus monoceros*; **2c:** *Rhinolophus imaizumii*; **2d:** *Rhinolophus ferrumequinum*; **2e:** *Rhinolophus luctus*; **3a:** *Myotis hosonoi*; **3b:** *Myotis nattereri*; **4:** *Plecotus auritus*; **5:** *Barbastella leucomelas*; **6a:** *Pipistrellus endoi*; **6b:** *Pipistrellus javanicus*; **6c:** *Pipistrellus abramus*; **7:** *Murina leucogaster*; **8:** *Miniopterus schreibersii*.

large mitochondria whose end-to-end junctions are directly opposite on the dorsal and ventral aspects of the midpiece (Fig. III b, c). The two opposite mitochondria are conspicuously thick and ovoidal in *M. nattereri* and *M. macrodactylus* (Fig. III b), and rhombiform in *P. abramus* (Fig. III c).

In *Murina leucogaster*, in the Murininae, the sperm nucleus occupies 4.0 µm of the spatulate head (4.7 µm in length), and is about 2.0 µm in width (Fig. I 7a), being wedge-shaped in sagittal section and surmounted by a small acrosome (Fig. I 7b). The spermatozoon of this species resembles that of *Myotis* in morphology and in size. In *Miniopterus schreibersii*, in the

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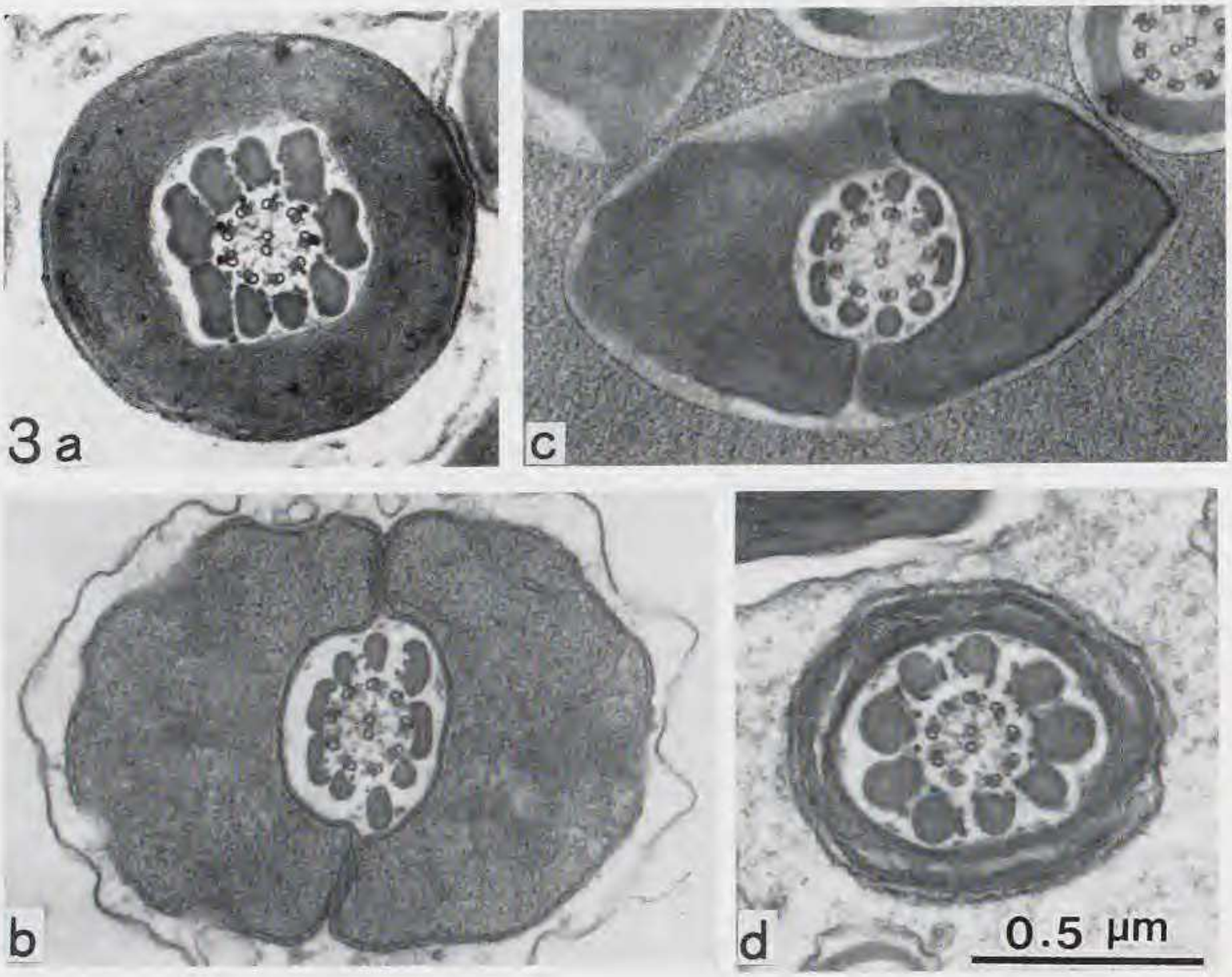

FIG. III. — Electron micrographs of the midpiece (transverse section). **a:** *Pteropus dasymallus*; **b:** *Myotis nattereri*; **c:** *Pipistrellus abramus*; **d:** *Miniopterus schreibersii*. In *Myotis nattereri* and *Pipistrellus abramus*, there are just two large mitochondria to each turn of the sheath: the end-to-end junctions of mitochondria are in line along the dorsal and ventral aspects of the midpiece.

Miniopterinae, the spermatozoon has a large, spatulate head. The nucleus occupies 5.0 µm of the head (8.0 µm in length), being about 2.8 µm in width (Fig.I 8a). When cut sagittally, the head is lanceolate, tapering to a tip (Fig. I 8b). The large acrosome, tipped with a swelling, protrudes anteriorly beyond the leading edge of the nucleus for a length of 3.0 µm. The midpiece in this species is very short compared with that in the other subfamilies examined, and mitochondria are conspicuously small in number and in size (Figs. II 8, 3d).

DISCUSSION

The spermatozoa of the two subfamilies examined, Pteropodinae and Macroglossinae in the Pteropodidae, belonging to the Megachiroptera, are similar in possessing a flattened, wide spoon-shaped head with convex lateral contours, a large acrosome, a rounded nucleus and a tail with a long midpiece. The fact that Pteropodidae share such similarities in sperm morphology with Soricidae which is a group of the basic insectivores retaining primitive characters within eutherians, strongly supported MILLER's concept [11] that Megachiroptera should be allocated to the lowest taxon within the Chiroptera from the viewpoint of adaptation for flight.

In Microchiroptera, the spermatozoon of *Emballonura furax*, belonging to the Emballonuridae, has a spatulate head according to the figure presented [6], although the authors do not refer to such a morphological character. The spermatozoa of Phyllostomatidae have a broad head as well as those of Megachiroptera, and are reasonably constant at the generic and specific levels [3], differing from those of Rhinolophidae and Vespertilionidae in morphology. In *Rhinolophus*, the sperm head, with a large acrosome. is uniform in morphology at the specific level. In *R. ferrumequinum*, the midpiece, with abundant mitochondria, is very long compared with the other four species examined. This species copulates in autumn, and the spermatozoa are stored in the female reproductive tract until the next spring (sperm storage type) [19]. Thus, the well-developed midpiece in *R. ferrumequinum* may be an adaptation for prolonged survival of the spermatozoa. The reason why the midpiece in the other four species of *Rhinolophus* is shorter than that in *R. ferrumequinum* is unknown as their reproductive patterns have not yet been examined in detail.

It has been suspected on the basis of morphological characters that the vespertilionid ancestor was a *Myotis*-like bat. According to ANDO [1], the karyotypes of *Myotis* are characterized by having a prototypic karyotype for the Vespertilionidae, and the karyotypes of Myotini and Plecotini (Myotini *s. lat.*), Pipistrellini and Nycticeini seem to be derived from the ancestral karyotype of the Vespertilionidae. As to *Myotis*, the prototypic karyotype is said to have remained unchanged at or even after divergence, and consequently their karyotypes resemble each other at the specific level. Also from sperm morphology, the spermatozoa of *Myotis* are similar in general structure at the specific level, showing a typical structure in Vespertilionidae in possessing a spatulate head and small acrosome. The morphology of the sperm heads is consistent in Myotini (*s. lat.*), thus being in good agreement with the phylogenetic relationships. However, there are great differences in number and in size of mitochondria; for example, the midpiece in *Myotis macrodactylus*, *M. nattereri* and *M. lucifugus* is very long, and their mitochondria are large in number and in size compared with the other three species of *Myotis*. It seems to be an adaptive phenomenon for prolonged sperm survival that the well-developed midpiece is present in *M. lucifugus* in which clear evidence has been obtained for the prolonged fertilizing life of the spermatozoon [21].

In Pipistrellini, taking distinct differences with regard to the karyotype and the baculum morphology between *Pipistrellus abramus abramus* and *P. javanicus javanicus* into consideration, they should be separated as a good species from each other, although they have been classified at a subspecific level, *P. a. abramus* and *P. a. javanicus* [1]. This assumption of specific distinctness is supported also from differences in fine structure of spermatozoa, particularly in number and in size of mitochondria. Further, the well-developed mitochondrial sheath in *P. pipistrellus* [16, 17] and *P. abramus* [7, 8, 20] with unusual reproductive habits (sperm storage type) may be concerned with an adaptation for prolonged survival of spermatozoa, as in *M. lucifugus* mentioned above. The sperm nucleus of Pipistrellini (*Pipistrellus*, *Nyctalus* and *Vespertilio*) is shorter than that of Myotini (*s. lat.*), and the above three genera are consistent in sperm morphology. As for Murininae, the subfamily has been regarded as a conservative taxon with primitive characters, being Myotini-like in sperm morphology.

In *Miniopterus schreibersii*, in the Miniopterinae, copulation is followed closely by ovulation in mid-October [12-15]. Embryonic development, however, proceeds very slowly during the long hibernation period (delayed implantation type) [9]. Correspondingly, the *M. schreibersii* spermatozoon differ conspicuously from the other subfamilies examined (Vespertilioninae, Murininae) in having a spatulate head surmounted by the remarkably large acrosome, and a very short midpiece whose mitochondria are conspicuously small in number and in size, compared with the other subfamilies. Such great differences in sperm morphology clearly reveal subfamilial distinctness. On the basis of the dental formula [10], it has been suggested that the genus *Miniopterus* be placed in a separate family Miniopteridae. The fact that the spermatozoon has the very short midpiece, mentioned above, may be associated with the fact that the spermatozoon does not need to retain its viability for as long as 5-7 months unlike the sperm storage type; thus, *M. schreibersii* is in a striking contrast to bats belonging to the sperm storage type with a well-developed midpiece.

In conclusion, the present study has demonstrated the potential usefulness of sperm morphology in taxonomic studies of Chiroptera. The presence of a well-developed midpiece (unique arrangement of abundant, large mitochondria) of spermatozoa in hibernating bats is explained as an adaptive phenomenon for prolonged survival of spermatozoa, and the details of the reproductive pattern in bats yet to be examined are needed to analyse adaptive implications of sperm morphology.

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